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[Title of the invention] PARTICLE-MOVING/FIXING DEVICE

[Abstract]

[Problem to be Solved]

To provide a particle-moving/fixing device capable of obtaining a sufficient dielectrophoretic power for moving/fixing the particles.

[Solution]

This particle-moving/fixing device 2 is provided by forming a particle-housing part 16 at a base material 21 for constituting the particle moving/fixing device, and installing a surface electrode 31 at the surface side of the base material 21 and in the vicinity of the particle-housing part 16, and a reverse surface electrode 41 at the reverse surface side of the base material 21 and in the vicinity of the particle-housing part 16. By performing the passing of electricity to the electrodes 31, 41, the particles 12 are moved or fixed by the dielectrophoretic power caused by an electric field generated in a medium 13. The tips of the electrodes 31, 41 are arranged in a

state of extending out in the central part direction of the particle-housing part 16.

[Claims for the Patent]

[Claim 1]

A particle-moving/fixing device comprising a base material in which a particle-housing part for containing a particle floating in a medium is formed, a surface electrode provided on the surface side of said base material and in the vicinity of said particle-housing part, and a reverse surface electrode provided on the reverse surface side of said base material and in the vicinity of said particle-housing part, in which an electric field is generated in the medium caused by energization of said electrodes so that the particle is moved or fixed by a dielectrophoretic power by the electric field, characterized in that a tip of said electrode is arranged in a state extending in the center part direction of said particle-housing part.

[Claim 2]

The particle-moving/fixing device according to claim 1, wherein said base material is a glass plate, said particle-housing part is a penetrating part penetrating the glass plate in the thickness direction, and said surface electrode is a conductive metal plate joined to the surface side of the glass plate and in the vicinity of the penetrating part.

[Claim 3]

The particle-moving/fixing device according to claim 1 or 2, wherein said base material is a glass

plate, said particle-housing part is a penetrating part penetrating the glass plate in the thickness direction, said reverse surface electrode is formed on the surface side of an auxiliary glass plate for forming an electrode, and the auxiliary glass plate is attached to the reverse surface side of the glass plate.

[Claim 4]

The particle-moving/fixing device according to any one of claims 1 to 3, wherein a feeding path is provided at said base material, said particle-housing part is arranged on the feeding path, and a feeding electrode for generating an electric field in the feeding path is provided.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to a cell-moving/fixing device for moving or fixing a particle such as a cell in a medium.

[0002]

[Conventional Art]

An art called microinjection has been known in recent years as a micro-manipulation technology for manipulating a micro object in a particle state.

[0003]

The microinjection refers to a method of injecting DNA, RNA, organelle, various proteins, various medical

solutions and the like into a cell using an injection needle called micropipette made by machining a glass tube, for example. Such an art has drawn attention particularly recently as an effective method that can selectively introduce a specific gene and the like into a cell. Such method has an advantage that introduction efficiency is higher and more inexpensive than similar methods such as laser injection and the like.

[0004]

In the above art, since a liquid is injected into a cell in a state where a micropipette is stuck into a cell floating in a medium, the cell should be fixed by some means at penetration. If a gene or the like is to be introduced into a specific portion in a cell, the cell should be fixed in a state where the portion is directed to a predetermined direction where the pipette can be stuck easily. In the past, a pipette for fixing separate from a micropipette for sticking is used so as to suck a cell to its tip for fixing the cell.

[0005]

However, since the prior art method that the cell is sucked and fixed takes labor and time, it is difficult to expect improvement in productivity. As an alternative to that, the inventors have reached a unit provided with a cell-moving/fixing device 61 as shown in Figure 6. In this figure, the cell-moving/fixing device 61 is supported in a mounting holder 63 also

serving as a container in which a medium 62 is filled. At a center part of a glass plate 64 as a base material constituting the device 61, a cell chamber 66 for containing a cell 65 one by one is provided. In this figure, the cell chamber 66 as a particle-housing part is in a substantially bowl-like recess portion formed on the surface side of the glass plate 64. On the surface side of the glass plate 64 and in the vicinity of the cell chamber 66, a plurality of surface electrodes 67a are provided by sputtering and the like. On the reverse surface side of the glass plate 64 and in the vicinity of the cell chamber 66, a plurality of reverse surface electrodes 67b are provided also by sputtering and the like. The electrodes 67a, 67b are connected to a pad 69 through a wiring pattern 68, and a lead wire for power supply, not shown, is also connected to each pad 69. The device 61 is fixed onto a stage 71 of a microscope with the holder 63. Below the stage 71, an objective lens 72 is arranged, while above the stage 71, a micropipette 73 is arranged.

[0006]

Therefore, when a high-frequency voltage is applied to each of the electrodes 67a, 67b through each lead wire, an electric field is generated in the cell chamber 66, and the electric field gives a suitable dielectrophoretic power to the cell 65. Thus, after the cell 65 is moved in the cell chamber 66 for

position modification, the cell 65 can be fixed. Therefore, while observing the cell 65 from below the device 61, an operator can manipulate the micropipette 73 and stick its tip into the cell 65.

[0007]

[Problems to be Solved by the Invention]

However, with the configuration of the device 61 shown in Figure 6, since a distance between the surface electrode 67a and the cell 65 or a distance between the reverse surface electrode 67b and the cell 65 becomes longer, a sufficient dielectrophoretic power for moving or fixing the cell 65 can not be obtained. Also, between the surface electrode 67a and the reverse surface electrode 67b, the glass plate 64 with a specific permittivity smaller than that of water as the medium 62 is interposed. This makes one of factors that the sufficient dielectrophoretic power can not be obtained.

[0008]

Moreover, since the device 61 is not provided with any structure for carrying-in/carrying-out of the cell 65 with respect to the cell chamber 66, the operator should perform such work carefully using the micropipette 73, which is extremely cumbersome.

[0009]

The present invention was made in view of the above problems and has a first object to provide a

particle-moving/fixing device that can obtain a sufficient dielectrophoretic power to move/fix a particle.

[0010]

A second object of the present invention is to provide a particle-moving/fixing device that can perform carrying-in/carrying-out work of a particle easily.

[0011]

[Means for Solving the Problems]

In order to solve the above problems, an invention described in claim 1 is a particle-moving/fixing device comprising a base material in which a particle-housing part for containing a particle floating in a medium is formed, a surface electrode provided on the surface side of the base material and in the vicinity of the particle-housing part, and a reverse surface electrode provided on the reverse surface side of the base material and in the vicinity of the particle-housing part, in which an electric field is generated in the medium caused by energization of the electrodes so that the particle is moved or fixed by a dielectrophoretic power by the electric field, a tip of the electrode is arranged in a state extending in the center part direction of the particle-housing part.

[0012]

An invention described in claim 2 is characterized

in that in claim 1, the base material is a glass plate, the particle-housing part is a penetrating part penetrating the glass plate in the thickness direction, and the surface electrode is a conductive metal plate joined to the surface side of the glass plate and in the vicinity of the penetrating part.

[0013]

An invention described in claim 3 is characterized in that in claim 1 or 2, the base material is a glass plate, the particle-housing part is a penetrating part penetrating the glass plate in the thickness direction, the reverse surface electrode is formed on the surface side of an auxiliary glass plate for forming an electrode, and the auxiliary glass plate is attached to the reverse surface side of the glass plate.

[0014]

An invention described in claim 4 is characterized in any one of claims 1 to 3, a feeding path is provided at the base material, the particle-housing part is arranged on the feeding path, and a feeding electrode for generating an electric field in the feeding path is provided.

[0015]

An "operation" of the present invention will be described below. According to the invention described in claim 1, a distance between the tip of the electrode extending in the center part direction of the particle-

housing part and the particle floating in the medium in the particle-housing part becomes shorter. In addition, a thickness of the base material interposed between the tip of the surface electrode and the tip of the reverse surface electrode is surely reduced. Therefore, if an electric field is generated in the medium by electrifying the electrode, the sufficient dielectrophoretic power to move or fix the particle can be obtained.

[0016]

According to the invention described in claim 2, since the penetrating part penetrating the glass plate in the thickness direction is made the particle-housing part, there is no base material interposed between the tip of the surface electrode and the tip of the reverse surface electrode. Thus, the sufficient dielectrophoretic power can be surely obtained. Since the surface electrode made of a conductive metal plate is provided with strength to some degree, the tip can be easily extended to the center part direction of the particle-housing part. That is, the device can be manufactured relatively easily.

[0017]

According to the invention described in claim 3, since the penetrating part penetrating the glass plate in the thickness direction is made the particle-housing part, there is no base material interposed between the

tip of the surface electrode and the tip of the reverse surface electrode. Thus, the sufficient dielectrophoretic power can be surely obtained. Also, by having a structure in which the auxiliary glass plate with the reverse surface electrode formed is attached, the tip of the reverse surface electrode can be easily extended to the center part direction of the particle-housing part. That is, the device can be manufactured relatively easily.

[0018]

According to the invention described in claim 4, by generating an electric field in the feeding path by electrifying the feeding electrode, the particle can be moved along the feeding path. Therefore, carrying-in/carrying-out work of a particle into the particle-housing part can be performed easily.

[0019]

[Embodiments of the Invention]

A cell manipulation system 1 of an embodiment embodying the particle-moving/fixing device of the present invention will be described below in detail referring to Figures 1 to 5.

[0020]

This cell manipulation system 1 comprises a cell-moving/fixing device 2, a mounting holder 3, an objective lens 4 as lower-part observing means, a stage 5 of a microscope, a micropipette 6, a manipulator (not

shown), a light source (not shown) and the like. The cell-moving/fixing device 2 and the mounting holder 3 constitute a single cell-moving/fixing unit UN1.

[0021]

As shown in Figure 1, at the center part of the stage 5, a through hole 11 is provided. Below the through hole 11, the objective lens 4 is disposed in an upward state. The objective lens 4 can be moved in a perpendicular direction by manipulating manipulating means, not shown. The light source is arranged above the through hole 11 with a distance. The micropipette 6 is arranged on an upper side of the stage 5. The micropipette 6 is produced by drawing a heated and molten glass tube thinly. A tip portion of the micropipette 6 is formed in a sharpened shape so that it can stick a plant cell 12 with a diameter of several tens to several hundreds μm . The micropipette 6 is driven in a three-dimensional manner by manipulating a manipulator. Driving methods of the manipulator include hydraulic method, air method, mechanical method and the like, for example. A base end portion of the micropipette 6 is connected to a pressurizer such as syringe, not shown. From the pressurizer, solution containing DNA and the like, for example, is press-fed.

[0022]

As shown in Figure 1, the mounting holder 3 is mounted by fastening of a bolt and the like, not shown,

to the center part of an upper face of the microscope stage 5 with the through hole 11. The holder 3 is a member with a bottom in the circular shape and has a through hole formed at the center of the bottom portion. The upper face of the bottom portion in the holder 3 supports a lower face side of an outer periphery part of the cell-moving/fixing device 2. As a result, the device 2 is mounted in the holder 3 in a sandwiched state.

[0023]

The cell-moving/fixing device 2 of this embodiment is in a so-called multilayered structure in which two pieces of the base materials are bonded together. For convenience of microscopic observation, a base material having light translucency is preferably selected and in view of that, a glass base material is selected here. Among various types of glass, borosilicate glass with high transparency is particularly preferably selected. On the reverse surface side of a glass plate 21 as an upper base material, an auxiliary glass plate 22 as a lower base material is bonded through an adhesive, not shown. The adhesives such as a UV-curing adhesive and the like are used, for example.

[0024]

As shown in Figures 1 to 3, the thickness of the glass plate 21 is approximately 100 to 200 μm and its planar shape is a square. A size of a side of the

square is set at approximately 20 to 30 mm. The glass plate 21 is provided with a cell chamber 16 as a particle-housing part for containing the plant cell 12 floating in a culture fluid. The cell chamber 16 of this embodiment is specifically a penetrating hole penetrating the glass plate 21 in the thickness direction. The cell chamber 16 shown in Figure 2 has a non-circular shape and equal sectional area and is located at the center part of the glass plate 21. In such cell chamber 16, the plant cell 12 as a particle is contained one by one. The cell chamber 16 may be provided in plural for the single glass plate 21.

[0025]

As shown in Figure 2, the glass plate 21 is provided with a feeding groove 23 as a feeding path. The feeding groove 23 of this embodiment is specifically a penetrating groove penetrating the glass plate 21 in the thickness direction. The feeding groove 23 in Figure 2 extends in a straight state while passing through the center part of the glass plate 21, and the cell chamber 16 is arranged in the middle of it. A right end portion of the feeding groove 23 in Figure 2 is an inlet part 24 for supplying a culture fluid 13 into the feeding groove 23. A left end portion thereof is an outlet part 25 for discharging the culture fluid 13 from the feeding groove 23. In the case of this embodiment, the cell chamber 16 is located exactly at

the intermediate point between the inlet part 24 and the outlet part 25. A width of the feeding groove 23 preferably becomes wider from the cell chamber 16 toward the inlet part 24 or the outlet part 25. That is because since the inlet part 24 or the outlet part 25 becomes wider, work to supply the plant cell 12 into the feeding groove 23 and the like is facilitated.

[0026]

The cell chamber 16 and the feeding groove 23 are preferably formed by laser machining. That is because laser machining can form a fine hole/groove easily and accurately as compared with etching and the like, for example. It is needless to say that spot-facing, drilling, grinding, etching and the like may be performed instead of laser machining.

[0027]

On the surface side of the glass plate 21 and in the vicinity of the cell chamber 16, a plurality of (four, here) surface electrodes 31 for driving a particle is joined. In this embodiment, a plate material made of conductive metal such as copper, aluminum and the like is used as the surface electrode 31. The conductive metal plates preferably have a shape having at least one corner part such as a polygon, for example. In this embodiment, a square-shaped conductive metal plate (5 mm × 5 mm) is used.

[0028]

As shown in Figure 2, a tip of each surface electrode 31 (that is, one of four corner parts in the single surface electrode 31) is arranged, extending in the center part direction of the cell chamber 16. Therefore, in a region below the tip of the extending surface electrode 31, there is no thick glass plate 21 but a relatively large space is provided (See Figure 1). The extending amount of the tip is set at approximately 0.1 to 1 mm.

[0029]

Each of the surface electrodes 31 is entirely covered by an insulating layer (layer made of a transparent insulating resin material, for example) R in order to prevent electrolysis caused by contact with water. However, a surface of the corner part located at the outer periphery part of the glass plate 21 in the surface electrode 31 is exposed to the outside from the insulating layer R. To each of these lead extraction part, a lead wire 33 is joined using a conductive adhesive 32. In order to improve insulation properties, the conductive adhesive 32 is further covered entirely by a resin layer 34. In the case of this embodiment, four lead wires 33 connected to the upper face side of the glass plate 21 is pulled out to the outside of the holder 3 through an upper opening. The pulled-out lead wire 33 is electrically connected to a device (not shown) generating a high-frequency AC

voltage of several 10 kHz to several MHz.

[0030]

As shown in Figure 2, at the surface center part of the auxiliary glass plate 22 attached on the reverse surface side of the glass plate 21, a plurality of (four, here) reverse surface electrodes 41 for driving a particle are formed. Each reverse surface electrode 41 in this embodiment has an elongated square shape. A tip (inner end) of each reverse surface electrode 41 is arranged in a state extending to the center part direction of the cell chamber 16. Therefore, in a region above the tip of the extending reverse surface electrode 41, there is no thick glass plate 21 but a relatively large space is provided. The extending amount of the tip is set at approximately 0.1 to 1 mm similarly to that of the surface electrode 31.

[0031]

At four locations on the outer periphery part of the auxiliary glass plate 22, a rectangular connection pad 42 is formed. All the connection pads 42 are arranged outside the glass plate 21. Each connection pad 42 and the corresponding reverse surface electrode 41 are electrically connected to each other through a linear pattern 43. The linear pattern 43 is constituted by four straight portions 43a with different lengths and each straight portion 43a is connected to each other at an angle of 90°. The linear

pattern 43 can be also grasped that it starts a base end (outer end) of the reverse surface electrode 41 as a start point, advances counterclockwise in Figure 2, bends at a right angle three times and then, connects to the connection pad 42 as an end point. Each of the four straight portions 43a of the linear pattern 43 is arranged so as to keep a positional relation in parallel with each other with an equal interval. Each linear pattern 43 is disposed crossing the feeding groove 23 at two locations. More specifically, each linear pattern 43 crosses the feeding groove 23 once between the inlet part 24 and the cell chamber 16 and crosses the feeding groove 23 once again between the cell chamber 16 and the outlet part 25.

[0032]

This cell-moving/fixing device 2 is also provided with a feeding system for feeding a particle such as the plant cell 12 by a dielectrophoretic power. That is, in the device 2 of this embodiment, in addition to the feeding groove 23, a feeding electrode E1 for generating an electric field in the feeding groove 23 is provided. In this device 2, the above linear pattern 43 substantially plays a role of the feeding electrode E1. In other words, a conductive region connecting the reverse surface electrode 41 and the connection pad 42 to each other functions as the feeding electrode E1.

[0033]

The feeding electrode E1 is provided in plural (here, the number of the electrodes is four similarly to the reverse surface electrode 41), and they are preferably disposed in a state with a predetermined angle to a direction in which the feeding groove 23 extends (more preferably, in a state orthogonal to the direction as in Figures 2 and 3).

[0034]

Here, formation procedures of the reverse surface electrode 41, the connection pad 42 and the feeding electrode E1 (linear pattern 43) will be described in brief. First, the auxiliary glass plate 22 for forming electrode is prepared. In this embodiment, the circular auxiliary glass plate 22 with a diameter of 40 mm and a thickness of approximately 100 to 200 μm is used. An area of this auxiliary glass plate 22 is larger than that of the glass plate 21. On the surface side of such auxiliary glass plate 22, a chrome layer and a gold layer are formed in this order by a conventional known vacuum deposition method. As a result, the reverse surface electrode 41, the connection pad 42, and the feeding electrode E1 with the thickness of approximately several μm and in the double-layer structure are obtained.

[0035]

Next, a transparent insulating film 44 is formed

on the whole surface side of the auxiliary glass plate 22. The reason why such insulating film 44 is formed is to prevent electrolysis of water when a voltage is applied by separating the reverse surface electrode 41 from the culture fluid 13. The thickness of the insulating film 44 is preferably set at approximately several μm . Such thin insulating film 44 can be formed by spin-coating SOG, for example. If the insulating film 44 is too thick, there is a fear that it makes a negative factor for reduction of a distance between the electrodes 31, 41. At a spot corresponding to each of the connection pads 42 in the insulating film 44, an opening portion 45 for pulling-out lead is provided. In a post process, to the connection pad 42 exposed from the opening portion 45, the lead wire 33 is joined, respectively, using the above-mentioned conductive adhesive 32. In order to improve insulation properties, the conductive adhesive 32 is further covered entirely by the resin layer 34.

[0036]

Next, the auxiliary glass plate 22 on which the insulating film 44 covering the reverse surface electrode 41 is formed is attached to the reverse surface side of the glass plate 21 using the above adhesive. As a result, the reverse surface electrode 41 is substantially provided on the reverse surface side of the glass plate 21 and in the vicinity of the

cell chamber 16.

[0037]

The four lead wires 33 joined to the connection pad 42 are pulled out to the outside of the holder 3 through the upper opening. The pulled-out lead wire 33 is electrically connected to a device generating a high-frequency AC voltage of several 10 kHz to several MHz.

[0038]

Here, for convenience of explaining the positional relation of the electrodes, reference characters U1, U2, U3, U4 are given to the surface electrodes 31 and reference characters D1, D2, D3, D4 are given to the reverse surface electrodes 41 (See Figures 2, 3). It is known that electrodes U1-D1, U2-D2, U3-D3, U4-D4 are in the corresponding vertical positional relation, respectively.

[0039]

Figures 4(a) to 4(c) show tables for explaining energization of these eight electrodes 31, 41 (U1 to U4, D1 to D4). If the plant cell 12 is to be rotated in an arrow A1 direction in Figure 2 around the axis extending in the longitudinal direction of the feeding groove 23, it is only necessary to apply a positive voltage to specific two electrodes 31 (41) as U1 + U2 → U3 + U4 → D3 + D4 → D1 + D2. At this time, the electrodes other than the above specific two electrodes

31 (41) are connected to GND (ground). By performing energization in the order of $D1 + D2 \rightarrow D3 + D4 \rightarrow U3 + U4 \rightarrow U1 + U2$, the plant cell 12 can be rotated in a reverse direction around the above axis.

[0040]

If the plant cell 12 is to be rotated to the right around a straight line parallel with the thickness direction of the device 2, it is only necessary to apply a positive voltage to only specific single electrode 31 as $U1 \rightarrow U2 \rightarrow U3 \rightarrow U4$ sequentially clockwise. At this time, the electrodes other than the above specific single electrode 31 are connected to GND. If this is performed sequentially in the reverse order as $U4 \rightarrow U3 \rightarrow U2 \rightarrow U1$, the plant cell 12 can be rotated to the left around the straight line.

[0041]

If the plant cell 12 is to be raised, a positive voltage is applied to U1 and U2, while a negative voltage is applied to U3 and U4 and the others are connected to GND. If the plant cell 12 is to be lowered, it is only necessary that a positive voltage is applied to U1 to U4, while D1 to D4 are connected to GND. If the plant cell 12 is not to be moved but fixed, it is only necessary that a voltage value to be applied to U1 to U4, D1 to D4 is fixed. That is, it is only necessary that the same voltage is applied to both the electrodes 31, 41.

[0042]

The above operation control is possible because if a high-frequency voltage is applied to the electrodes 31, 41 through the lead wire 33, an electric field is generated in the cell chamber 16, and the electric field brings about a suitable dielectrophoretic power to the plant cell 12. In this case, since the permittivity of the plant cell 12 as a particle object is different from the permittivity of the culture fluid 13, which is a medium in general, when the electric field acts, electrostatic polarization is generated in the plant cell 12. As a result, the suitable dielectrophoretic power is generated in the cell chamber 16, and the electrostatically polarized plant cell 12 is drawn to the stronger electric field.

[0043]

Next, a feeding system in this embodiment will be described based on Figure 5. For convenience of explanation, the feeding electrode located on the outermost peripheral side in the device 2 is referred to as "E11", the one located immediately inside E11 as "E12", the one located immediately inside E12 as "E13", and the one located on the innermost side as "E14".

[0044]

In Figure 5(a), the plant cell 12 is located on the feeding electrode E11, and no voltage is applied yet to each of the electrodes E11 to E14 at this time.

Therefore, there is no electric field generated in the feeding groove 23.

[0045]

A positive voltage is applied to the electrode E12 in this state and the electrode E11 is connected to GND. Then, by an action of the dielectrophoretic power brought about by the generated electric field, the plant cell 12 is moved onto the electrode E12 (See Figure 5(b)). Next, the positive voltage is applied to the electrode E13 and the electrode E12 is connected to GND. Then, similarly by the action of the dielectrophoretic power, the plant cell 12 is moved onto the electrode E13 (See Figure 5(c)). By sequentially performing the above voltage application, the plant cell 12 can be moved along the feeding groove 23 and finally the plant cell 12 can reach the inside of the cell chamber 16.

[0046]

Next, an example of a specific usage of the system 1 configured as above will be described. The holder 3 is set on the microscope stage 5 and the cell-moving/fixing device 2 is fixed on the upper face of the bottom portion in the holder 3. In this state, the feeding groove 23 and the cell chamber 16 are filled with the sterilized culture fluid 13. As a result, insides of the feeding groove 23 and the cell chamber 16 are both brought into an environment with the

culture fluid 13, and extinction of the plant cell 12 susceptible to drying can be prevented. The plant cell 12 may be a single cell such as protoplast, pollen and the like or may be a cell group (that is, multiple cells) including specific organs such as germ, callus and the like.

[0047]

An operator drives a culture-fluid supply device, not shown, so as to generate a flow in the culture fluid 13 and introduces the plant cell 12 in the inlet part 24 of the carrying-in groove 23. By applying a voltage to the feeding electrode E1 as above, the feeding system is driven. As a result, the plant cell 12 is moved along the feeding groove 23 and transferred into the cell chamber 16. Next, the operator applies a high-frequency voltage to the electrodes 31, 41 while conducting microscopic observation and modifies the position of the plant cell 12. In this case, if a portion into which a specific gene is to be introduced is a germ, for example, adjustment is made so that the germ part is directed to the tip portion of the micropipette 6.

[0048]

Next, the operator drives and manipulates the micropipette 6 while observing the microscope and advances its tip slowly toward the plant cell 12, which is a target. After the operator confirms by the

microscope that the tip portion of the micropipette 6 is stuck into the germ of the plant cell 12, the operator slowly presses a head portion of a syringe so as to push out liquid with DNA inside to the outside. Then, the liquid is discharged from the tip portion via the micropipette 6. Therefore, the DNA can be injected into the germ of the plant cell 12. After the above introduction processing is finished, the operator needs to retreat the micropipette 6 and remove the tip portion from the plant cell 12. As a result, the specific gene can be selectively and efficiently introduced into the plant cell 12. After that, the plant cell 12 into which the gene has been introduced is moved again through the feeding groove 23 by driving of the feeding system and transferred to the outlet part 25. By driving the culture fluid supply device, the plant cell 12 is discharged to the outside of the device 2.

[0049]

Therefore, according to this embodiment, the following effects can be obtained:

(1) In this device 2, each surface electrode 31 is arranged in a state where its tip at the corner part is extended in the center part direction of the cell chamber 16. Also, each reverse surface electrode 41 is arranged similarly in the state where the tip at the corner part is extended in the center part direction of

the cell chamber 16. Therefore, a distance between the tips of the extended electrodes 31, 41 and the plant cell 12 floating in the culture fluid 13 in the cell chamber 16 is surely shorter than that of the conventional structure. In addition, the thickness of the glass interposed between the tip of the surface electrode 31 and the tip of the reverse surface electrode 41 becomes surely smaller. Therefore, when an electric field is generated in the culture fluid 13 by energization of the electrodes 31, 41, a sufficient dielectrophoretic power to move or fix the plant cell 12 can be obtained.

[0050]

(2) In this device 2, the cell chamber 16 penetrating the glass plate 21 in the thickness direction is provided as a particle-housing part at the glass plate 21. As a result, such a state is brought about that there is almost no glass with a specific permittivity smaller than that of water interposed between the tip of the surface electrode 31 and the tip of the reverse surface electrode 41. Though the insulating film 44 is present between the surface electrode 31 and the reverse surface electrode 41, it is extremely thinner than the glass plate 21 and almost negligible. Thus, according to the configuration of this embodiment, the sufficient dielectrophoretic power can be surely obtained. Also, the surface electrode 31

made of the conductive metal plate is provided with a strength enough to bear at least its own weight. Thus, its tip can be extended easily in the center part direction of the cell chamber 16, and even in that case, the tip would hardly deform or sag. That is, the device 2 of this embodiment is in a structure that can be manufactured relatively easily.

[0051]

(3) In this device 2, the auxiliary glass plate 22 on which the reverse surface electrode 41, the connection pad 42, and the feeding electrode E1 are formed is attached to the glass plate 21 in the structure. The tip of the reverse surface electrode 41 can be easily extended in the center part direction of the cell chamber 16. That is, the device 2 in this embodiment has a structure that can be manufactured relatively easily.

[0052]

Also, since the reverse surface electrode 41 and the like can be formed at the auxiliary glass plate 22 in advance, it is not necessarily required to use a material such as a conductive metal plate and the like, but the thin reverse surface electrode 41 and the like can be formed easily by a usual thin film forming method.

[0053]

(4) In this device 2, the feeding groove 23 having

the cell chamber 16 is provided on the path, and the feeding electrode E1 for generating an electric field in the feeding groove 23 is provided. Therefore, the suitable dielectrophoretic power is generated by energization of the feeding electrode E1, and by the power, the plant cell 12 can be moved along the feeding groove 23. Therefore, the carrying-in work of the plant cell 12 into the cell chamber 16 and the carrying-out work of the plant cell 12 from the cell chamber 16 can be performed easily.

[0054]

(5) The feeding groove 23 in this embodiment extends in one direction and does not bend in its entirety. In addition, each feeding electrode E1 crosses the direction in which the feeding groove 23 extends. Therefore, there is a small possibility that the plant cell 12 collides against the side wall of the feeding groove 23 or is brought into sliding contact when the plant cell 12 is being moved. Thus, there is an advantage that damage on the plant cell 12 is minimized and its yield is surely improved.

[0055]

Moreover, in the device 2, the linear pattern 43 connecting the reverse surface electrode 41 and the connection pad 42 to each other also functions as the feeding electrode E1. Thus, the connection pad 42 or the lead wire 33 for energizing the reverse surface

electrode 41 can be also used as the one for energizing the feeding electrode E1. Therefore, space saving and structure facilitation can be achieved.

[0056]

The embodiment of the present invention may be changed as follows:

- Instead of using the linear pattern 43 connecting the reverse surface electrode 41 and the connection pad 42 to each other also as the feeding electrode E1, the feeding electrode E1 may be provided separately.

[0057]

- The particle-housing part 16 and the feeding path 23 do not necessarily have to penetrate through the glass plate 21. However, the penetration structure as in the embodiment can be machined and formed by laser more easily and more suitable from the viewpoint of permittivity.

[0058]

- As the method of forming the reverse surface electrode 41 and the like on the surface side of the auxiliary glass plate 22, not only the vacuum deposition exemplified in this embodiment but sputtering, ion-plating, CVD, PVD, plating, printing and the like, for example, may be employed.
- As a material forming the glass plate 21 and the auxiliary glass plate 22, those other than borosilicate glass such as silica glass and the like may be used,

for example. Not limited to glass, transparent ceramic materials represented by zirconia and the like may be selected as a forming material of the base material.

[0059]

- The number, shape, layout and the like of the electrodes 31, 41 can be changed arbitrarily as long as it does not depart from the gist of the present invention.

- The feeding electrode E1 and the feeding groove 23 may be omitted if they are not particularly required.

[0060]

- Not only that the culture fluid 13 for plant illustrated in the embodiment is used as the medium, mere distilled water containing little nutrient and the like may be used as the medium. It is needless to say that the medium can be changed as appropriate according to the type of a particle.

[0061]

- The system 1 of the present invention can be used not only in introduction of the DNA itself into the plant cell 12 as described in the embodiment but also for various purposes. For example, if the particle object is an animal egg cell, the system can be used for injection of sperm into egg cytoplasm (egg cytoplasm sperm injection method) and the like, one of microinsemination technologies. It is needless to say that the system 1 of the present invention can be used

not only in manipulation of a living substance such as the cell 12 and the like as an object but also in the manipulation of a non-living substance as the object.

[0062]

Next, in addition to the technical idea described in the claims, the technical idea to be grasped in the above-mentioned embodiment will be shown below with its advantages:

(1) In the particle-moving/fixing device provided with a base material in which a particle-housing part for containing a particle floating in a medium is formed and an electrode provided in the vicinity of the particle-housing part, in which an electric field is generated in the medium by energization of the electrode so that the particle is moved or fixed by a dielectrophoretic power caused by the electric field, the particle-moving/fixing device is characterized in that the base material is provided with a feeding system for feeding the particle by the dielectrophoretic power. Therefore, according to the invention described in this technical idea 1, the particle-moving/fixing device that can perform carrying-in/carrying-out work of the particle easily can be provided.

[0063]

(2) In the particle-moving/fixing device provided with a base material in which a particle-housing part

for containing a particle floating in a medium is formed and an electrode provided in the vicinity of the particle-housing part, in which an electric field is generated in the medium by energization of the electrode so that the particle is moved or fixed by a dielectrophoretic power caused by the electric field, the particle-moving/fixing device is characterized in that a feeding path communicating with the particle-housing part and a feeding electrode for generating an electric field in the feeding path are provided at the base material. Therefore, according to the invention described in the technical idea 2, the particle-moving/fixing device that can perform carrying-in/carrying-out work of the particle easily can be provided.

[0064]

(3) In claim 4, the technical ideas 1, 2, the feeding electrode is provided in plural and they are disposed in a state having an angle to the direction in which the feeding path extends.

[0065]

(4) In claim 4, the technical ideas 1, 2, the feeding electrode is provided in plural and they are disposed so as to cross the feeding path.

(5) In claim 4, the technical ideas 1, 2, the feeding electrode is provided in plural and they cross the direction in which the feeding path extends at a

right angle. Therefore, according to the invention described in the technical idea 5, the particles hardly collide against/slide on the side wall of the feeding path, by which damage on the particle is minimized and yield is improved.

[0066]

[Advantages of the Invention]

As described above in detail, according to the invention described in claim 1, the particle-moving/fixing device that can obtain a sufficient dielectrophoretic power to move/fix the particle can be provided.

[0067]

According to the invention described in claims 2 and 3, though the sufficient dielectrophoretic power can be surely obtained, the device can be manufactured relatively easily. According to the invention described in claim 4, the particle-moving/fixing device that can perform carrying-in/carrying-out work of the particle easily can be provided.

[Brief Description of the Drawings]

[Figure 1]

Figure 1 is an outline sectional diagram illustrating a cell manipulation system using a unit provided with a cell-moving/fixing device of an embodiment embodying the present invention.

[Figure 2]

Figure 2 is a plan view of the cell-moving/fixing device of the embodiment.

[Figure 3]

Figure 3 is a plan view for explaining a pattern shape of an electrode formed on an auxiliary glass plate constituting the cell-moving/fixing device.

[Figure 4]

Figures 4(a) to 4(c) are tables for explaining voltage application patterns to each electrode of the cell-moving/fixing device.

[Figure 5]

Figures 5(a) to 5(c) are enlarged plan views of an essential part for explaining a feeding system provided at the cell-moving/fixing device.

[Figure 6]

Figure 6 is an outline sectional diagram illustrating a cell manipulation system using a conventional cell-moving/fixing device.

[Description of Symbols]

- 2 particle-moving/fixing device
- 12 plant cell as a particle
- 13 culture fluid as medium
- 16 penetrating part as particle-housing part (cell chamber)
- 21 glass plate as base material
- 22 auxiliary glass plate for forming electrode
- 23 feeding groove as feeding path

31 (U1 to U4) conductive metal plate as surface
electrode

41 (D1 to D4) reverse surface electrode

E1 feeding electrode

Figure 4(a)

#1 (U1 TO U4: SURFACE ELECTRODE, D1 TO D4: REVERSE
SURFACE ELECTRODE)

#2 ROTATION

Figure 4(b)

RIGHT ROTATION

Figure 4(c)

#1 RAISE

#2 LOWER

よって前記媒質中に電界を発生させ、その電界のもとら誘電泳動力により前記粒子を移動または固定させるようにした粒子移動/固定装置において、前記基材には、前記粒子収容部に連通する搬送路と、その搬送路内に電界を発生させる搬送用電極とが設けられていることを特徴とする粒子移動/固定装置。従って、この技術的思想2に記載の発明によれば、粒子の搬入・搬出作業を容易に行うことができる粒子移動/固定装置を提供することができる。

【0064】(3) 請求項4、技術的思想1、2において、前記搬送用電極は複数であって、それらは前記搬送路の延びる方向に対して角度を持った状態で配設されていること。

【0065】(4) 請求項4、技術的思想1、2において、前記搬送用電極は複数であって、それらは前記搬送路を横切るように配設されていること。

(5) 請求項4、技術的思想1、2において、前記搬送用電極は複数であって、それらは前記搬送路の延びる方向に対して直交していること。従って、この技術的思想5に記載の発明によれば、粒子が搬送路の側壁に衝突・擦接しにくくなるため、粒子の損傷が最小限に抑えられ、収率が向上する。

【0066】

【発明の効果】以上詳述したように、請求項1に記載の発明によれば、粒子を移動・固定させるのに十分な誘電泳動力を得ることができる粒子移動/固定装置を提供することができる。

【0067】請求項2、3に記載の発明によれば、十分

な誘電泳動力を確実に得ることができるにもかかわらず、比較的簡単に製造することができる。請求項4に記載の発明によれば、粒子の搬入・搬出作業を容易に行うことができる粒子移動/固定装置を提供することができる。

【図面の簡単な説明】

【図1】本発明を具体化した実施形態の細胞移動/固定装置を備えるユニットを利用したセルマニピュレーションシステムを示す概略断面図。

【図2】実施形態の細胞移動/固定装置の平面図。

【図3】細胞移動/固定装置を構成する補助ガラス板上に形成された電極のパターン形状を説明するための平面図。

【図4】(a)～(c)は、細胞移動/固定装置の各電極に対する電圧印加パターンを説明するための表。

【図5】(a)～(c)は、細胞移動/固定装置の備える搬送システムを説明するための要部拡大平面図。

【図6】従来の細胞移動/固定装置を利用したセルマニピュレーションシステムを示す概略断面図。

【符号の説明】

2…粒子移動/固定装置、12…粒子としての植物細胞、13…媒質としての培養液、16…粒子収容部としての貫通部(細胞チャンバ)、21…基材としてのガラス板、22…電極形成用の補助ガラス板、23…搬送路としての搬送溝、31(U1～U4)…表面電極としての導電性金属板、41(D1～D4)…裏面電極、E1…搬送用電極。

【図1】 FIG. 1

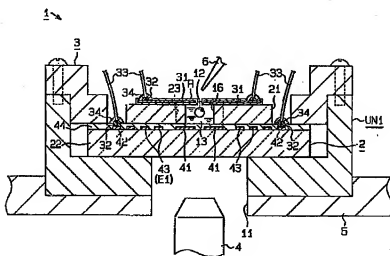


Fig. 2
【図2】

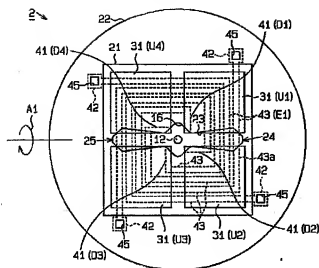


Fig. 3
【図3】

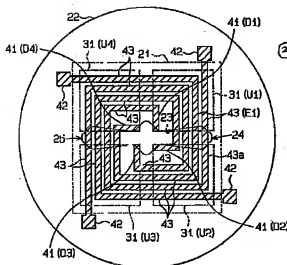


Fig. 4
【図4】

(a) ① U1~U4: 絶縁層, D1~D4: 導電層

②

	U1	U2	U3	U4	D1	D2	D3	D4
面1	+	+	0	0	0	0	0	0
	0	0	+	+	0	0	0	0
	0	0	0	0	0	0	+	+
	0	0	0	0	+	+	0	0

(b)

	U1	U2	U3	U4	D1	D2	D3	D4
面1	+	0	0	0	0	0	0	0
	0	+	0	0	0	0	0	0
	0	0	+	0	0	0	0	0
	0	0	0	+	0	0	0	0

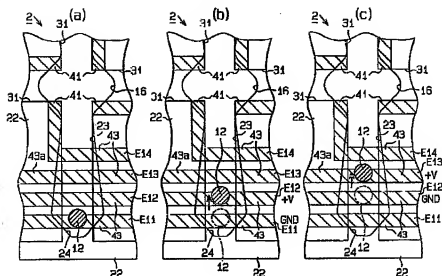
(c)

①

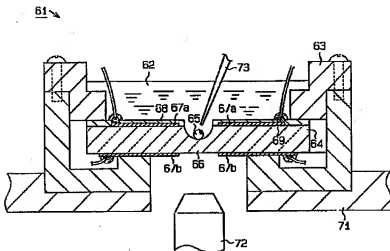
②

	U1	U2	U3	U4	D1	D2	D3	D4
上層	+	+	-	-	0	0	0	0
下層	+	+	+	+	0	0	0	0

【图5】 FIG.5



【図6】 FIG. 6



フロントページの続き

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